

10/090807

order refs

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100 cells. The pipette was also used to scrape and remove differentiated areas of the colonies. The PBS was then changed to regular pre-equilibrated human stem cells medium containing dispase (Gibco) 10mg/ml and incubated for 5-10 minutes (at 37°C, 5% CO₂). As soon as the clumps were detached they were
5 picked up by wide bore micro-pipette, washed in PBS containing Ca and Mg and transferred to a fresh feeder layer.

e) Human stem cell cryopreservation.

Early passage cells were cryo-preserved in clumps of about 100 cells by
10 using the open pulled straw (OPS) vitrification method (Vajta et al 1998) with some modifications. French mini-straws (250µl, IMV, L'Aigle, France) were heat-softened over a hot plate, and pulled manually until the inner diameter was reduced to about half of the original diameter. The straws were allowed to cool to room temperature and were then cut at the narrowest point with a razor
15 blade. The straws were sterilised by gamma irradiation (15-25 K Gy). Two vitrification solutions (VS) were used. Both were based on a holding medium (HM) which included DMEM containing HEPES buffer (Gibco, without sodium pyruvate, glucose 4500mg/L) supplemented with 20% fetal bovine serum (Hyclone, Logan, Utah). The first VS (VS1) included 10% dimethyl sulfoxide
20 (DMSO, Sigma) and 10% ethylene glycol (EG, Sigma). The second vitrification solution (VS2) included 20% DMSO, 20% EG and 0.5M sucrose. All procedures were performed on a heating stage at 37°C. 4-6 clumps of ES cells were first incubated in VS1 for 1 minute followed by incubation in VS2 for 25 seconds. They were then washed in a 20µl droplet of VS2 and placed within a droplet of
25 1-2µl of VS2. The clumps were loaded into the narrow end of the straw from the droplet by capillary action. The narrow end was immediately submerged into liquid nitrogen. Straws were stored in liquid nitrogen. Thawing was also performed on a heating stage at 37°C as previously described with slight modifications (Vajta et al 1998). Three seconds after removal from liquid
30 nitrogen, the narrow end of the straw was submerged into HM supplemented with 0.2M sucrose. After 1 minute incubation the clumps were further incubated 5 minutes in HM with 0.1M sucrose and an additional 5 minutes in HM.

27. A differentiated cell produced by the method according to any one of claims 19 to 26.

28. A differentiated cell according to claim 27 which is a somatic cell
5 selected from the group including a committed progenitor cell capable of self renewal or differentiation into one or several somatic lineages, or a fully mature somatic differentiated cell.

29. A cell line HES-1.

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30. A cell line HES-2.

31. A fibroblast cell strain which is highly suitable for the promotion of embryonic stem cell growth and the inhibition of extraembryonic differentiation.

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32. A fibroblast cell strain according to claim 31 derived from the inbred mouse strains 129/Sv, CBA or the cross of 129/Sv and C57/Bl6.

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33. A method of preserving a differentiated or undifferentiated cell wherein the cells undergo vitrification.

34. A method according to claim 33 wherein the vitrification is Open Pulled Straw (OPS) vitrification.

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35. A method of preventing and treating a congenital disease, said method including:

obtaining an undifferentiated stem cell according to claim 18;

introducing a genetic modification to the congenital disease; and

inducing differentiation to a somatic cell line capable of transplantation to

30 a patient in need.

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